Research Article Open Access

Mariia Nesterkina, Ulrich R. Bernier, Nurhayat Tabanca, Iryna Kravchenko*

Repellent activity of monoterpenoid esters with neurotransmitter amino acids against yellow fever mosquito, *Aedes aegypti*

https://doi.org/10.1515/chem-2018-0015
received October 2, 2017; accepted January 22, 2018.

Abstract: Repellent activity of monoterpenoid esters (**1**-**6**) with neurotransmitter amino acids (GABA and glycine) was investigated against *Aedes aegypti* by using a "cloth-patch" assay and compared to reference standard *N*,*N*-diethyl-3-methylbenzamide (DEET). Monoterpenoid esters showed repellent activity with minimum effective dosages (MED) in the range of 0.031-0.469 mg/cm^2. The carvacrol ester of GABA (**2**, MED of 0.031 ± 0.008 mg/cm^2) exhibited the highest repellency of six monoterpenoid esters tested in comparison to the standard repellent DEET (MED of 0.009 ± 0.002 mg/cm^2); however, the repellent activity of carvacrol-glycine ester (**5**) decreased 4-fold compared to the carvacrol-GABA derivative (**2**). The repellent activities of menthol GABA (**1**, MED= 0.375 ± 0.000 mg/cm^2) and glycine ester (**4**, MED=0.312 ± 0.063 mg/cm^2) were similar. The guaiacol-glycine ester (**6**) was 3.75-fold more efficacious than the guaiacol ester of GABA (**3**). In the present study, we report repellent efficacy of prolonged exposure to GABA and glycine esters of menthol, carvacrol, guaiacol (**1**-**6**) as compared to the repellent activities of their monoterpene moieties alone.

Keywords: repellents, mosquito, monoterpenoids, GABA, glycine

***Corresponding author: Iryna Kravchenko,** Department of Organic and Pharmaceutical Technology, Odessa National Polytechnic University, 65044 Odessa, Ukraine; Department of Pharmaceutical Chemistry, I.I. Mechnikov Odessa National University, 65026 Odessa, Ukraine, E-mail: kravchenko.pharm@gmail.com
Mariia Nesterkina: Department of Organic and Pharmaceutical Technology, Odessa National Polytechnic University, 65044 Odessa, Ukraine; Department of Pharmaceutical Chemistry, I.I. Mechnikov Odessa National University, 65026 Odessa, Ukraine
Ulrich R. Bernier: USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608, USA
Nurhayat Tabanca: USDA-ARS, Subtropical Horticulture Research Station, 13601 Old Cutler Rd., Miami, FL 33158 USA

1 Introduction

Mosquitoes represent one of the most significant threats to human and veterinary health throughout the world. The mosquito *Aedes aegypti* (L.) is a primary carrier of viruses causing dengue fever, dengue hemorrhagic fever and yellow fever in the tropical and subtropical regions of the world; an estimated 2-2.5 billion people are at risk globally for dengue [1]. Recently the World Health Organization (WHO) recognized that *Aedes*-borne Zika virus has generated an international public health emergency [2,3]. Personal protection is one method to prevent viral transfer through mosquito bites. In particular, chemical agents such as *N*,*N*-dimethyl-3-methylbenzamide (DEET) have been used effectively to repel mosquitoes for more than 75 years [4]. Importantly, despite long-term and widespread use, public concerns remain over the toxicity of DEET and other chemical repellents, and experimental results suggest that DEET indeed does exert negative effects on human health [4].

Insect pest control technology today focuses upon the development of "green" methods [5], and there are several non-DEET products available on the market [6]. Picaridin [1-piperidinecarboxylic acid, 2(2-hydroxyethyl)-1-methyl propylester, also referred to as KBR 3023] has been added to the list of repellents recommended by the U.S. Centers for Disease Control and Prevention (CDC). The compound IR3535 (3-[*N*-butyl-*N*-acetyl]-aminopropionic acid, ethyl ester) has been registered as an active ingredient and sold as an insect repellent [6]. Medicinal plant essential oils have been extensively studied for their abilities to repel or kill insects [7,8]. Most of these botanical repellents contain citronella, eucalyptus, geraniol and/or cedarwood oil as the active ingredient [6]. Two citronella oil-based repellents (*Cymbopogon nardus* and *C. winterianus*) are registered and sold in commercial preparations [6]. Lemon eucalyptus oil (*Corymbia citriodora*) containing *p*-menthane-3,8-diol as the main active component has also been added to the list of recommended repellents [6]. Plant essential oils are generally recognized as an important natural source of

monoterpenoids, which are well known to demonstrate repellent, antifeedant and insecticidal properties against insects [1,9].

Monoterpenoids have been studied as candidate insecticides for many years; however, their mode of action is not yet fully understood [10]. Monoterpenoids act on various targets in the insect nervous system, including γ-aminobutyric acid (GABA)-gated chloride channels, octopamine receptors, tyramine receptors, acetylcholine esterase, nicotinic acetylcholine receptors (nAChR), sodium channels, and possibly other [11,12]. Different monoterpenoids have been found to bind to ionotropic GABA receptors in insects. Thymol, linalool, menthol, camphor, carvone, borneol, and other monoterpenoids have been shown to be positive allosteric modulators of insect GABA receptors [13].

One of the impediments to the use of pure terpenes (or terpenoids) is that they may cause skin irritation or allergic reactions and they evaporate quickly from the surface of the skin. As an approach to overcoming these obstacles, we propose to use terpenoid esters containing fatty or amino acids to reduce the side effects of pure terpenoids and to prolong the action of repellents. In this study, we investigated the repellent efficacy of three monoterpenoids (menthol, carvacrol and guaiacol) and their esters combined with amino acids (GABA and glycine).

2 Experimental

2.1 General

Monoterpenoid esters of GABA and glycine were synthesized via Steglich esterification as described [14,15]. The synthesized compounds are designated numbers as follows: (*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl 4-aminobutyrate hydrochloride (**1**), 5-isopropyl-2-methylphenyl 4-aminobutyrate hydrochloride (**2**), 2-methoxyphenyl 4-aminobutyrate hydrochloride (**3**), (*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl aminoacetate hydrochloride (**4**), 5-isopropyl-2-methylphenyl aminoacetate hydrochloride (**5**), 2-methoxyphenyl aminoacetate hydrochloride (**6**).

Menthol (CAS Registry No. 1490-04-6, Sigma-Aldrich, St. Louis, MO, USA), carvacrol (CAS Registry No. 499-75-2, Sigma-Aldrich, St. Louis, MO, USA) and guaiacol (CAS Registry No. 90-05-1, TCI America research Chemicals, Portland, OR, USA) were obtained commercially.

2.2 Mosquitoes

Mosquitoes used in all bioassays were female *Aedes aegypti* (Orlando strain, 1952) from the colony maintained at the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE,USDA-ARS) in Gainesville, FL. Pupae were obtained from the onsite colony and maintained in laboratory cages until ready for use in experiments. Newly emerged mosquitoes were maintained *ad libitum* on a 10% sucrose solution at 25-28°C ambient temperature, 60-80% relative humidity and a 14:10 (light:dark) photoperiod. Nulliparous female mosquitoes aged 6-10 d were preselected for host-seeking behavior from stock cages using a hand draw-box and a collection trap [16].

2.3 Cloth Patch Assay

Repellent efficacy was determined as the minimum effective dosage (MED) of a compound against *Ae. aegypti* as described [17,18]. Briefly, monoterpenoids or their esters were dissolved in acetone to obtain 25 and 2.5 μmol/cm^2 of each compound on a 50 cm^2 cloth that was immersed into the prepared solutions. The cloth was then dried approximately 3 min before applying to a volunteer's arm, which was inserted into a cage containing mosquitoes. The arm was protected by a plastic glove and sleeve in which window was cut and covered with the treated cloth. To estimate repellent activity, the protected arm was inserted into the mosquito cage for 1 min and the numbers of blood-feeding mosquitos and insects that remained biting through the cloth were determined. In order to assess the MED value, the cloth was impregnated with a series of following dosages in acetone: 1.5, 0.75, 0.375, 0.094, 0.047, 0.023 and 0.011 mg/cm^2.

Ethical approval: Written informed consent was obtained for all human subjects used in this study in accordance with protocol #636-2005, as approved by the University of Florida Institutional Review Board (IRB-01).

3 Results and Discussion

Among botanically-derived insecticides, terpenoids are often considered prime candidates because they typically have lower toxicity to mammals, relatively high volatilities and potent toxicities to pests [19]. Although DEET is widely used as an active ingredient in a variety of repellent sprays, liquids, impregnated materials, etc., the toxicity and environmental consequences of DEET-based

products continue to be of concern to some consumers. Herein, we explore repellent candidates with expected mechanism of repellency that differ from that of DEET. One of the insecticidal mechanisms is associated with modulation of GABA-gated chloride receptors [13]. Some monocyclic terpenoids have been found to be positive allosteric modulators at insect GABA receptors [13]. Thus, the combination of terpenoid residues with GABA in single molecules is potentially an attractive approach for pest control. Moreover, GABA co-releases with another neurotransmitter amino acid, glycine, and co-activation of glycine and GABA receptors has been extensively described [20]. Thus, in the present work, esters based on terpenoid alcohols (menthol, carvacrol, and guaiacol) and amino acids (GABA, glycine) have been synthesized as potential repellents with prolonged activity.

Synthesis of esters based on monocyclic terpenoids with GABA or glycine (**1**–**6**) was carried out via Steglich esterification with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as a catalyst in dichloromethane (**Figure 1**) [14,15].

In our research, DEET, known as a highly efficacious repellent, served as a positive control (MED value of 0.009 ± 0.002 mg/cm^2) (**Table 1**). As the MED value of DEET may vary slightly for different sets of experiments, an additional index – repellency proportion (RP) – was introduced. The RP represents the ratio of the MED for the candidate to the MED for DEET and was determined for a particular set of replicates. The investigation of insecticidal activity of synthesized compounds against *Aedes aegypti* demonstrated that most of the terpenoid esters produced MED values that ranged from 0.125 - 0.469 mg/cm^2; this range implicates these compounds as weaker repellents compared to DEET.

The most toxic derivative tested against *Aedes aegypti* was carvacrol ester **2** with a repellent MED of 0.031 mg/cm^2. Carvacrol acylation with GABA residues leads to a reduction in repellent activity compared with pure carvacrol (MED = 0.013 ± 0.005 mg/cm^2) indicating a significant role for the free OH group in the terpenoid structure. The influence of a hydroxyl group on the insecticidal action was also demonstrated for compounds with alkylated OH groups. Carvacrol methyl ethers and thymol methyl ether are inferior repellents compared to the pure terpenoids, with MED values of 0.063 ± 0.016 and 0.258 ± 0.117 mg/cm^2, respectively [22, 23]. Interestingly, carvacrol and thymol demonstrated good repellency whereas the biological precursor of carvacrol and thymol, *p*-cymene, which is devoid of the hydroxyl group, did not repel at the highest test concentration of 1.5 mg/cm^2 [22]. A comparison of MED values for pure menthol (0.094 ± 0.000 mg/cm^2) and guaiacol (0.109 ± 0.041 mg/cm^2) to MEDs of their corresponding esters (0.312 mg/cm^2 for ester **4** and 0.125 mg/cm^2 for ester **6**) also suggests that the hydroxyl group is necessary for the repellent activity.

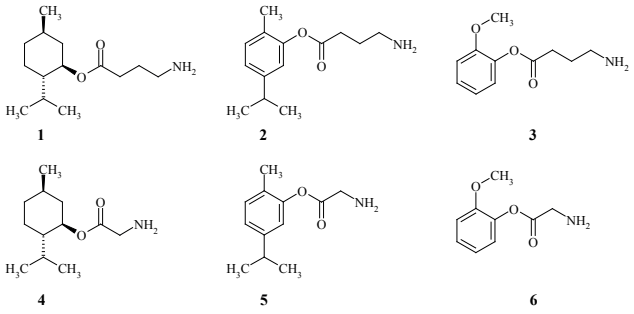

Figure 1: Structures of terpenoid esters containing GABA (**1**-**3**) and glycine (**4**-**6**) residues. All esters were prepared as hydrochlorides.

Table 1: Minimum effective dosage (MED) of tested esters against *Aedes aegypti*.

Compound	MED, mg/cm^2 ± SEM	MW	RP*
1	0.375 ± 0.000	277.5	41.667
2	0.031 ± 0.008	271.5	3.444
3	0.469 ± 0.282	245.5	52.111
4	0.312 ± 0.063	249.5	34.667
5	0.125 ± 0.031	243.5	13.889
6	0.125 ± 0.031	217.5	13.889
Menthol	0.094 ± 0.000	156	8.545
Carvacrol**	0.013 ± 0.005	150	2.167
Guaiacol	0.109 ± 0.041	124	9.909
DEET	0.009 ± 0.002	191	-

*RP – ratio of compound MED to DEET MED for a particular experiment.
** Results from our previous study [21]

Here, we have demonstrated the possibility of developing contact repellents based on a combination of terpenoid esters and neurotransmitter amino acids. However, their potential utility as spatial repellents will be a subject of examination in the next phase of our research. Since these derivatives undergo hydrolysis resulting in a gradual release of the terpenoid moiety, such a mechanism could provide prolonged insecticidal action.

4 Conclusions

In the present study, we evaluated the repellent activity of monoterpenoid esters combined with GABA and glycine against *Ae. aegypti*. Among the derivatives, the carvacrol ester of GABA was the most active repellent

with a MED value of 0.031 ± 0.008 mg/cm^2. The presence of a free hydroxyl group in the terpenoid structure plays a significant role in the manifestation of the insecticidal effect. For the practical use of terpenoid derivatives as long lasting repellents, further research confirming their prolonged action is needed.

Disclaimer: No potential conflict of interest was reported by the authors.

Acknowledgements: This study was partly funded by the Deployed War-Fighter Protection Research Program via grants from the U.S. Department of Defense through the Armed Forces Pest Management Board. We thank Natasha M. Agramonte, Greg Allen and Erin OReilly (USDA-ARS, CMAVE, Gainesville, FL) for mosquito repellent bioassays.

References

[1] Kumar S., Wahab N., Warikoo R., Bioefficacy of Mentha piperita essential oil against dengue fever mosquito *Aedes aegypti* L., Asian Pac. J. Trop. Biomed., 2011, 2, 85-88.

[2] de Melo M.C., Donalisio M.R., de Freitas A.R.R., Zika virus from discovery to the present days: Bibliometric review study, Int. J. Curr. Res., 2017, 9, 46728-46740.

[3] Islam J., Zaman K., Duarah S., Raju P.S., Chattopadhyay P., Mosquito repellents: An insight into the chronological perspectives and novel discoveries, Acta Trop., 2017, 167, 216-230.

[4] Frances S.P., Efficacy and safety of repellents containing deet, In: Debboun M., Frances S.P., Strickman D. (Eds.), Insect repellents principles, methods and uses, CRC Press, Boca Raton, FL., 2007.

[5] Maia M.F., Moore S.J., Plant-based insect repellents: a review of their efficacy, development and testing, Malar. J., 2011, 10, 1-14.

[6] Xue R-de., Ali A., Day J.F., Commercially available insect repellents and criteria for their use, In: Debboun M., Frances S.P., Strickman D. (Eds.), Insect repellents principles, methods and uses, CRC Press, Boca Raton, FL., 2007.

[7] Ayvaz A., Sagdic O., Karaborklu S., Ozturk I., Insecticidal activity of the essential oils from different plants against three stored-product insects, J. Insect. Sci., 2008, 10, 1-10.

[8] Benelli G., Flamini G., Canale A., Cioni P.L., Conti B., Toxicity of some essential oil formulations against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), Crop Prot., 2012, 42, 223-229.

[9] Pumnuan J., Insung A., Fumigant toxicity of plant essential oils in controlling thrips, Frankliniella schultzei (Thysanoptera: Thripidae) and mealybug, Pseudococcus jackbeardsleyi (Hemiptera: Pseudococcidae), J. Ent. Res., 2016, 40, 1-10.

[10] Park B.S., Choi W.S., Kim J.H., Kim K.H., Lee S.E., Monoterpenes from Thyme (Thymus vulgaris) as potential mosquito repellents, J. Am. Mosq. Control Assoc., 2005, 21, 80-83.

[11] Coats J.R., Karr L.L., Drewes C.D., Toxic and neurotoxic effects of monoterpenoids in insects and earthworms, In: Hedin P. (Ed.), Natural Occuring Pest Bioregulators, ACS Symposium series, Washington, D.C., 1991.

[12] Kostyukovsky M., Rafaeli A., Gileadi C., Demchenko N., Shaaya E., Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests, Pest Manag. Sci., 2002, 58, 1101-1106.

[13] Tong F., Investigation of mechanisms of action of monoterpenoid insecticides on insect gamma-aminobutyric acid receptors and nicotinic acetylcholine receptors, PhD thesis, Iowa State University, Ames, USA, 2010.

[14] Nesterkina M., Kravchenko I., Synthesis and pharmacological properties of novel esters based on monocyclic terpenes and GABA, Pharmaceuticals, 2016, 13, 1-10.

[15] Nesterkina M., Kravchenko I., Synthesis and pharmacological properties of novel esters based on monoterpenoids and glycine, Pharmaceuticals, 2017, 10, 1-10.

[16] Posey K.H., Schreck C.E., An airflow apparatus for selecting female mosquitoes for use in repellent and attraction studies, Mosquito News, 1981, 41, 566-568.

[17] Schreck C.E., Posey K., Smith D., Repellent activity of compounds submitted by Walter Reed Army Institute of Research. Part I. Protection time and minimum effective dosage against *Aedes aegypti* mosquitoes, Tech. Bull., 1977, 1549.

[18] Katritzky A.R., Wang Z., Slavov S., Dobchev D., Hall C.D., Tsikolia M., et al., Novel carboxamides as potential mosquito repellents, J. Med. Entomol., 2010, 47, 924-938.

[19] Norris E.J., Coats J.R., Current and future repellent technologies: the potential of spatial repellents and their place in mosquito-borne disease control, Int. J. Environ. Res. Public Health, 2017, 14, 1-15.

[20] Jonas P., Bischofberger J., Sandkühler J., Corelease of two fast neurotransmitters at a central synapse, Science, 1998, 281, 419-424.

[21] Tabanca N., Bernier U.R., Agramonte N.M., Tsikolia M., Bloomquist J.R., Discovery of natural products, Curr. Org. Chem., 2016, 20, 2690-2702.

[22] Tabanca N., Bernier U.R., Ali A., Wang M., Demirci B., Blythe E.K., et al., Bioassay-guided investigation of two Monarda essential oils as repellent of yellow fever mosquito against *Aedes aegypti*, J. Agric. Food Chem., 2013, 61, 8573-8580.

[23] Tabanca N., Bernier U.R., Tsikolia M., Becnel J.J., Sampson B., Werle C., et al., Eupatorium capillifolium essential oil: Chemical composition, antifungal activity, and insecticidal activity, Nat. Prod. Commun., 2010, 5, 1409-1415.